EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF ETHANOLIC EXTRACT OF SEENTHIL CHURANAM

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MARCH – 2010.

CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF ETHANOLIC EXTRACT OF SEENTHIL CHURANAM IN MICE AND RATS"** submitted by Dr. T.A.R. RAJA in partial fulfillment of the requirements for the award of Degree of Doctor of Medicine in Pharmacology by The Tamilnadu Dr. M. G. R Medical University, Chennai is a bonafide record of work carried out by him in the Institute of Pharmacology, Madras Medical College, Chennai during the academic year 2009-2010.

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CONTENTS

INTRODUCTION

INTRODUCTION

Man has been waging a constant battle against innumerable diseases right from the day of his existence on earth. During the course of his search for the proper kind of weapons to fight against agonizing ailments, he realized that the plant kingdom was one of the richest armamentaria which could provide the appropriate tools for his well being. With the development of traditional systems of medicines like Siddha, Unani, Ayurveda and Homeopathy, the herbal plants are being sought after, both by patients and by clinicians in search for cure of diseases[1].

Alleviation of pain and Inflammation has always remained a prime concern of medicine. Willow barks that contain salicin have long been used to treat pain and inflammation. Such usage led to the synthesis of salicylic acid in 1860 and aspirin in 1899. Opioids, the most potent pain killers known to date were also derived from a plant source Papaver somniferum. Thus both narcotic and non-narcotic analgesics have had their origin in herbal medicine [2].

Synthetic substitutes have no doubt taken over, but none of the analgesic and anti-inflammatory drugs available today can be considered ideal. The search should therefore continue and it is felt that herbal medicine has still a lot in store to be unearthed by diligent effort.

PAIN:

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain can be categorized as nociceptive or neuropathic pain. Nociceptive pain is one which is associated with injury such as burns or a broken bone. Neuropathic pain is one which usually occurs due to the changes in the nervous system. Pain of neuropathic origin is described in terms of abnormal sensations (eg. heat, burning sensation, cold and numbness).

With many pathological conditions, tissue injury is the immediate cause of pain and this results in the local release of variety of chemical agents, which are assured to act on the nerve terminals, either activating them directly or enhancing their sensitivity to other forms of stimulation[3].

Analgesics are those drugs that provide pain relief. They are classified as Narcotic and Non-narcotic analgesics. Other drugs, notably the tricyclic antidepressants and anti-epileptics such as gabapentin, have been used to relieve pain, particularly neurologic pain, but are not routinely classified as analgesics.

They provide symptomatic relief, but have no effect on the cause [4].

INFLAMMATION:

Inflammation (Latin-to set on fire) is the response to an injurious stimulus. It can be evoked by a wide variety of noxious agents, eg; infections, antibodies or physical injuries. Inflammatory responses occur in three distinct temporal phases, each apparently mediated by different mechanisms.

1. An acute phase characterized by transient local vasodilatation and increased capillary permeability.

2. A delayed, sub acute phase characterized by infiltration of leucocytes and phagocytic cells.

3. A chronic proliferative phase, in which tissue degeneration and fibrosis occur [5].

Metabolites of arachidonic acid are the main mediators playing a pivotal role in inflammation. Inhibition of these products is the main pathway by which most anti-inflammatory agents act [4].

The wide spread use of anti-inflammatory drugs in chronic diseases such as rheumatoid arthritis, osteoarthritis and ankylosing spondylitis has evoked the extensive search for new drugs with this property. Though we have standard analgesic and anti-inflammatory drugs like aspirin, indomethacin, phenylbutazone etc., because of their side effects, there is

extensive search for new drugs with less side effects. Search for newer analgesic and anti-inflammatory agents having better or at least equal efficacy with minimal side effects is continuing throughout the world [6].

As a result, a search for other alternatives seems necessary and beneficial. A large number of Indian medicinal plants are attributed with various pharmacological activities because they contain a diversified class of phytochemicals. India has an extensive forest cover, enriched with plant diversity and several plants are being used in Indian traditional medicine. Medicinal plants have a wide variety of chemicals from which novel analgesic and anti-inflammatory agents could be discovered [7].

ROLE OF TRADITIONAL MEDICINE:

India has an ancient heritage of traditional medicine. Materia medica of India provides lot of information on the folklore practices and traditional aspects of therepeutically important natural products[8].

During the last decade, in many developed countries there has also been a growing interest in herbal medicine, acupuncture and alternative systems of medicine. Consequently, an increase in international trade in herbal medicines and other types of traditional medicines has occurred. Indian traditional medicine is based on various systems including Ayurveda, Siddha and Unani. The evaluation of these drugs is mostly based on phytochemical, pharmacological and allied approaches

including various instrumental techniques like chromatography, microscopy and others.

Plant products have traditionally provided most of the drugs in use. Despite the achievements of synthetic chemistry and the advances towards rational drug design, natural products continue to be essential in providing medicinal compounds [8].

SIDDHA SYSTEM OF MEDICINE :

The term siddha comes from "siddhi "-means perfection. This system is almost akin to Ayurvedha. It is an ancient traditional system of medicine developed by 18 siddhars who glorified human beings as the highest form of birth and believed that preserving human body is essential to achieve external bliss [9].

MODERN CONCEPTS IN MEDICINE:

In almost all the traditional systems of medicine the quality control aspect has been considered from the inception itself by the Rishis and later by the Vaidyas and Hakins. However, in modern concept it requires necessary changes in their approach. For the quality control of traditional medicine, the traditional methods are procured, studied, documented and then the traditional information about identification and quality assessment is interpreted properly in terms of modern assessment.

Quality assurance is an integral part of traditional medicine, which ensures that it delivers the required quality of medicaments. Today quality assurance is the thrust area for traditional formulations like churnas, bhasmas, liquid lehas etc., In olden days these traditional medicinal formulations were prepared by vaidyas and were delivered to patients in the fresh form where quality assurance was not required.

Formulation of important guidelines in defining the extent and type of our participation is important while discussing the acceptance of the role of traditional systems of medicine and their practitioners in the primary health care. Some of the practices from traditional systems of medicine have already been explored and some requires special attention[10].

Therefore to establish the potentiality of traditional medicine, research needs to be simultaneously conducted on important aspects of these disciplines to meet the requirement of the society where they have to serve.

In siddha medicine, Seenthil churanam is traditionally claimed to have various medicinal properties. Seenthil churanam is a polyherbal formulation containing the extracts of Eclipta prostata, Tinospora cordifolia, and dried earthworm. Seenthil churanam is used in siddha medicine as an analgesic and anti-inflammatory agent in various conditions like sinusitis, hepatitis, rheumatism, gout, sprain etc.,. Though

Seenthil churanam is used in siddha medicine, no scientific work has been done on the churanam so far. Hence, this research work was taken up to scientifically evaluate and prove the analgesic and anti-inflammatory effects of Seenthil churanam.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Indian traditional medicine constitutes Ayurveda, Siddha and Unani. Siddha is practiced throughout the southern states of India. Siddha system of medicine is an integrated part of Indian system, which is very potent and unique system when compared with other traditional systems in existence. Siddha medicine means medicine that is perfect. Siddha medicine is claimed to revitalize and rejuvenate the dysfunctional organs that cause the disease. Drugs used in siddha medicine include metals, minerals, leaves, flowers, fruits and various roots of plants in a mixed basis[11].

Siddha drugs can be classified into three groups - thavaram [herbal product], thathu [inorganic substance] and jangamam [animal product][11]. Of these, herbal products play an important role in Siddha medicine. People from all continents have used hundreds to thousands of indigenous plants for the treatment of various diseases since prehistoric times. In the written record, the study of herbs dates back over 5000 years to the Sumerians, who described well established medicinal uses for plants. Indian traditional medicine has been using herbs such as turmeric possibly as early as 1900 B.C [12].

In the modern era, the use of herbs to treat disease is almost universal among many industrialized societies [13]. Many of the pharmaceuticals currently available to physicians have a long history of

use on herbal remedies, including opium, aspirin, digitalis and quinine. The WHO estimates that 80 percent of the world′s population presently uses herbal medicine for some aspect of primary health care [13]. Pharmaceuticals are prohibitively expensive for most of the world′s population, half of which lives on less than 2 U.S $ per day. In comparison, herbal medicines can be grown from seed or gathered from nature for little or no cost [14].

Since number of people, preferring natural health remedies and herbal health remedies are increasing day by day, Indian traditional medicine is gaining popularity all over the world. The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants. Herbal medicine is a major component in all traditional medicine systems, and a common element in Siddha, Ayurvedha, Homeopathy, Naturopathy, Traditional Chinese medicine and Native American medicine [13].

Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80 % show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived [15]. Atleast, 7000

medical compounds in the modern pharmacopoeia are derived from plants [15].

A few examples of herbal plants used in traditional medicine are,

- Aloe vera – used for the healing of burns and wounds.
- Allium sativum [garlic] – claimed to lower total cholesterol levels.
- Zingiber officinale [ginger] – claimed to improve GIT symptoms, may decrease nausea and vomiting of pregnancy.
- Naringenin [grape] – claimed to prevent Obesity.
- Camelia sinensis [green tea] – claimed to inhibit growth of breast cancer cells.
- Honey – claimed to reduce cholesterol, used in wound healing.
- Peppermint oil – claimed to have benefits for individuals with Irritable bowel syndrome.
- Pomegranate – claimed to inhibit cancer cell growth in mice[16].

In addition, many believe that herbal medicines are safe because they are natural. Herbal medicine may interact with synthetic drugs causing toxicity to the patient, herbal products may have contamination

that is a safety consideration and herbal medicines, without proven efficacy, may be used to replace medicines that have a proven efficacy [16].

Despite the increased popularity of herbal treatments, the safety and effectiveness of alternative medicines have not been scientifically proven and remain largely unknown. Proper animal studies and double blind clinical trials are needed to determine the safety and efficacy of each plant before they can be recommended for medical use. Furthermore, adulteration, inappropriate formulation, or lack of understanding of plant and drug interactions has led to various adverse effects [16].

In siddha medicine, Seenthil churanam is used for various diseases like Sinusitis, Osteoarthritis, Diabetes, Jaundice etc. It is used as an analgesic and anti-inflammatory drug in many conditions.

PAIN:

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. In many pathological conditions, tissue injury is the immediate cause of pain which results in the local release of a variety of chemical agents. These chemical agents are known to act on the nerve terminals, either activating them directly or enhancing their sensitivity to other forms of stimulation [17].

Pain is a major symptom in many medical conditions, and can significantly interfere with a person's quality of life and general functioning. Usually pain stops without treatment or responds to simple measures such as resting or taking an analgesic, and it is then called ‘acute’ pain. But it may also become intractable and develop into a condition called ‘chronic’ pain, in which pain is no longer considered a symptom but an illness by itself [17].

Nociceptive pain and neuropathic pain are the two main kinds of pain when the primary mechanism of production is considered. Nociceptive pain may be classified further in three types that have distinct organic origins and felt qualities.

1. Superficial somatic pain (or cutaneous pain) is caused by injury to the skin or superficial tissues. Cutaneous nociceptors terminate just below the skin and, due to the high concentration of nerve endings, produce a sharp, well-defined, localized pain of short duration. Examples of injuries that produce cutaneous pain include minor wounds and minor (first degree) burns.

2. Deep somatic pain originates from ligaments, tendons, bones, blood vessels and muscles. It is detected with somatic nociceptors. The scarcity of pain receptors in these areas produces a dull, aching, poorly-localized pain of longer duration than cutaneous pain; examples include sprains, broken bones, and myofascial pain.

3. Visceral pain originates from the viscera, or organs. Visceral nociceptors are located within body organs and internal cavities. The even greater scarcity of nociceptors in these areas produces pain that is usually more aching or cramping and of a longer duration than somatic pain. Visceral pain may be well-localized, but often it is extremely difficult to localize, and several injuries to visceral tissue exhibit "referred" pain, where the sensation is localized to an area completely unrelated to the site of injury [17].

Pain is part of the body's defense system, producing a reflexive retraction from the painful stimulus, and tendencies to protect the affected body part while it heals, and avoid that harmful situation in the future. Despite its unpleasantness, pain is an important part of animal life; in fact, it is vital to healthy survival and people with congenital insensitivity to pain have greatly reduced life expectancy. Pain is classified as acute or chronic [17].

Acute pain can last a few seconds or a few months but not more than six months. It lets the body know damage has occurred and something needs to be done to make it better. As healing of the injured areas occurs, the pain will normally decrease and eventually go away.

Chronic Pain is pain that lasts for more than six months. It is also known as persistent pain. It can be malignant, getting worse as the source of the pain worsens (as a tumor grows in cancer). It can also be non-

malignant as in a chronic illness such as arthritis. It can fluctuate over time with periods of severe pain followed by periods of no pain at all. Pain that does not stop when an injury is healed is also called chronic pain. Pain is not a normal part of aging and should be considered abnormal, and treated and ameliorated at any age [17].

TESTS FOR ANALGESIC ACTIVITY [18]:

Analgesics are evaluated for their central and peripheral activities. Many rodents, such as mice and rats are used for analgesic evaluation.

Centrally acting analgesics are evaluated by,

- Hot plate method
- Tail flick or radiant heat method
- Tail immersion test
- Haffner's tail clip method
- Formalin test

Peripherally acting analgesics are evaluated by,

- Acetic acid induced writhing test
- Randall – Selitto test
- Mechanical visceral pain model

INFLAMMATION:

Inflammation is the local reaction of vascularised living tissue to injury. The word inflammation is taken from the Latin word **"*Inflammatio*"** meaning burning. The inflammatory reactions, at first local, consisting primarily of changes in the blood vessels, the escape of cells and fluid from the blood into the tissues, and the consequent changes in the tissues[19] .

Agents that can produce inflammatory reaction are grouped as follows:

Mechanical	:	Crush and cut injuries.
Chemical	:	Corrosive acids, alkalies, phenol, body fluids such as bile and urine when they escape into the tissues.
Infections	:	Bacteria, fungi, virus and animal parasites.
Irradiation	:	Heat, ionizing radiation, light (particularly ultraviolet light).
Necrotic tissue	:	Infarcts.
Immunological	:	Antigen-antibody reaction [20].

TYPES OF INFLAMMATION:

Depending upon the duration and mode of onset, the inflammatory reaction can be classified as acute, sub-acute and chronic.

Acute inflammation is of relatively short duration lasting for minutes to several hours, and its main characteristics are exudation of fluid, plasma proteins (edema) and the migration of leucocytes, predominantly neutrophils.

Sub-acute inflammation lasts for one to six weeks (or more) and is usually seen in tubular structures like an appendix or fallopian tube. It is characterized by vascular exudative changes of acute inflammation and proliferative changes of chronic inflammation. Here the exudates consist chiefly of eosinophils, lymphocytes, plasma cells, histiocytes and fibroblasts [21].

Chronic inflammation, on the other hand, is of longer duration, lasting for weeks to months and is associated histologically with the presence of lymphocytes and macrophages and with proliferation of blood vessels and connective tissue.

Granulomatous inflammation is a specific type of chronic inflammation characterized by accumulation of modified macrophages (epitheloid cells) and initiated by a variety of infectious and noninfectious agents. The presence of poorly digestible irritants or T cell-mediated

immunity (with production of interferon) to the irritant, or both, appears to be necessary for granuloma formation [22].

MEDIATORS OF INFLAMMATION:

1] Histamine:

Histamine is released from mast cells by exocytosis during inflammatory or allergic reaction. In humans, histamine causes dilatation of arterioles and increases the vascular permeability of the venules (it however constricts large arteries). Inflammogenic activity of histamine is brought about mainly by H1 and partially by H2 receptors [23].

2] Serotonin:

Serotonin is an endogenous, biogenic amine found in gut-mucosa, enterochromaffin cells, platelets and in central nervous system. Release of serotonin from platelets is stimulated by platelet activating factor (PAF) and causes vasodilatation mediated through 5-HT1 receptors by release of nitric oxide from endothelial cells, inhibiting nor-adrenaline release from sympathetic nerve terminals and direct relaxant action on smooth muscles[24].

3] Bradykinin:

Bradykinin is a non-peptide which is 10 times more active vasodilator than histamine (on molar basis). Two types of Bradykinin

receptors are B1 and B2. B1 receptors which are present in normal vascular smooth muscle are up-regulated in inflammation [25].

4] Substance P:

It resembles bradykinin and is released by the nerve endings of sensory C-fibres. It degranulates mast cells and may be involved in the pathogenesis of the triple response [25].

5] Complement system:

Complement system plays a vital role in the inflammatory processes like vascular permeability, chemo taxis, opsonization prior to phagocytosis and lysis of target organisms. Various components of complement system are produced from two pathways viz. classic and alternate pathways [26].

6] Prostaglandins:

Prostaglandins (PGs) are generated at the site of inflammation through oxygenation, followed by cyclization of arachidonic acid to yield endoperoxide which inturn is converted to PGI_2 or thromboxane A_2 (TXA_2). It potentiates carrageenan induced edema and hyperalgesia in rats, while in rabbit skin it can induce hyperemia and augment plasma exudation in response to permeability inducing stimuli such as bradykinin [27].

7] Platelet activating factor:

PAF is an extraordinarily potent mediator of shock and inflammation. It exerts its biological effects by activating PAF receptor, consequently stimulating protein kinase C and increasing intracellular calcium ions [28].

8] Cytokines:

Cytokines involved in inflammation are interleukin IL-1, IL-2, IL-6, tumor necrosis factor (TNF) alpha and beta: interferon (IFN) alpha, beta and gamma, transforming growth factor (TGF) beta and chemokines [27].

TESTS FOR ANTI-INFLAMMATORY ACTIVITY [18]:

- Carragenan induced rat paw edema
- Croton oil edema in rats and mice
- Ultra Violet – erythema in guinea pigs
- Oxazolone - induced ear edema in mice
- Pleurisy test
- Granuloma pouch technique.

COMPOSITION OF SEENTHIL CHURANAM:

Eclipta prostrata

Tinospora cordifolia

Dried Earth worm

1] ECLIPTA PROSTRATA:[29]

Botanical source	:	Eclipta prostrata
Family	:	Asteraceae
Synonyms	:	Eclipta alba
	:	Eclipta erecta
	:	Verbesina alba
	:	Verbesina prostrata

OTHER NAMES:

Tamil	:	Karisilanganni
Hindi	:	Bhamgra
English	:	Trailing Eclipta, False daisy
Malayalam	:	Kannunni
Telugu	:	Galagara

DISTRIBUTION AND HABITAT:

It is a common plant and abundantly grows throughout India upto 6000ft height of hills. It is abundantly found in India, China, Brazil and United states.

GENERAL FEATURES:

It is a small and erect annual herb. Its stem is usually erect, flat or round, blackish green, profusely branched and pubiscent. Leaves are opposite, serrate, 3 – 5 cm long and blackish green in colour. Fruits are many seeded.

PARTS USED IN MEDICINE:

Whole plant.

MAJOR CHEMICAL COMPOUNDS FOUND IN THE PLANT:

Resins, ecliptine, nicotine, glycosides, alkaloids.

MEDICINAL USES: [29]

Anti-inflammatory, used in hepatotoxicity, abortion and miscarriage, uterine hemorrhage, piles, insect bites, stings, swellings and other skin diseases. Dried aerial parts are used as purgative, emetic in Arabic Countries, against snakebites in China, for diarrhea in India, for asthma in Thailand.

Fresh aerial part are used to treat snakebites in Brazil (Martz, 1992), for headaches, it is ground in sesame oil and applied to the forehead in India (Nagaraju and Rao, 1990), for common cold in Panama (Solís, 1995) Entire plant is used for tuberculosis and as haemostatic in China (Duke and Ayensu, 1985), for inflammation taken with black pepper and raw sugar in India (Jain, 1994), to treat wounds (caused by walking barefooted during rain) in Nepal (Manandhar, 1993), to treat vesicles on the skin, plant is crushed and soaked for an hour in water. Extract is applied to affected area in Somalia, to treat leprosy, plant is crushed and mixed with oil, mixture is applied to skin (Samuelsson, 1992) and to treat Diabetes mellitus in Taiwan (Lin, 1992).

Leaves are used to treat epilepsy in India, leaves are pounded with garlic and pepper if the patient is unconscious the extract is dropped into the nostril (Reddy, 1989), to treat stomach cancer mixed with Ageratum conyzoides, Spilanthes acmella, Vernonia conyzoides and jat, taken after meals in morning and evening in Indonesia (Hsu, 1967) and as an antiasthmatic, in colds, coughs, elephantiasis, hepatitis, splenitis, vertigo in Peru (Duke, 1994). Roots are used for insanity, four to five pills made from the root paste are given twice a day for seven days in India (Jain, 1994), for women after childbirth in Malaysia (Burkill, 1966), for jaundice, root plus seed of Ricinus communis are ground and paste is applied to eyes in India (Hemadri & Rao, 1984).

PHARMACOLOGICAL STUDIES:

Various biological activities have been reported in the literature for extracts of E. prostrata: SNC (Debelmas, 1976); hepatoprotective (Chandra, 1987; Singh, 1993; Sharma, 1991; Saxena, 1993); antiviral (Kusumoto, 1995, Zheng, 1988); antirheumatic (Dabral and Sharma, 1983); molluscicidal (Mendes, 1984); antimalarial (Misra, 1991) and antifertility (Misra, 1979).

The ether extract of the dried aerial parts showed antivenom effect, when administered 0.5 mg I.P. in mouse. The methanol extract (80%) of the dried aerial parts shows antihepatotoxic activity, administered in rat (1 mg/ml) (Kim and Park, 1994). The hydroalcoholic extract of the dried leaf reported analgesic activity, when administered intragastrically in mouse (100 mg/kg). The chloroform and methanol extract of the dried leaf (1 g/ml) showed antibacterial activity against Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus (Naovi, 1991; Phadke and Kulkarni, 1989).Several studies indicate that this plant is reported to have antiulcer [30], Immunomodulatory [31], analgesic [32], anti-inflammatory [33], anti-snake venom [34] and hypolipidemic [35] effects.

2] TINOSPORA CORDIFOLIA: [29]

Botanical source : Tinospora cordifolia

Family : Menispermaceae

Synonyms : Tinospora glabra , Cocculus cordifolius

OTHER NAMES:

Tamil	:	Seenthil
Telugu	:	Guduchi
Malayalam	:	chittamritam

DISTRIBUTION:

The climber is found throughout the tropical regions of India. It isalso found in Srilanka and China.

GENERAL FEATURES:

It is a large climbing shrub, often attains a great height and seems to be particularly fond of climbing up the trunks of large neem trees.

PARTS USED IN MEDICINE:

Stem, roots, leaves.

MAJOR CHEMICAL COMPOUNDS PRESENT:

Alkaloids, lactones, glycosides, steroids, polysaccharides.

MEDICINAL USE:

The leaves are beaten with honey and applied to ulcers. Dried and powdered fruit, mixed with ghee or honey, is used as a tonic and also in the treatment of jaundice and rheumatism. The root is powerful emetic

and used for visceral obstructions; its watery extract is used in leprosy. A decoction of the leaves is used for the treatment of gout, and young leaves, bruised in milk, are used as a liniment in erysipelas [29].

The whole plant is used in scabies in swine. The vine is used as an appetizer and for internal parasites in ruminants and for diarrhea in poultry. It is also used in stomach trouble. Stem, root and whole plant are used in sprain, abscess, tumour, wound, broken horn, cracked tail, anthrax, as a galactagogue and in the treatment of pneumonia, asthma, cough, swelling of lungs, colic, constipation, tetanus, pox and compound fracture [29].

PHARMACOLOGICAL STUDIES:

Analgesic and anti-inflammatory activity: [36] [37]

The whole plant extract of Tinospora cordifolia showed significant analgesic and anti-inflammatory activity in suitable animal models.

Anti-allergic activity: [38] [39]

An aqueous extract of Tinospara cordifalia decreased bronchospasm in guinea pigs, decreased capillary permeability in mice and reduced the number of disrupted, mast cells in rats.

Anti-cancer activity: [40]

A formulation containing Tinospora cordifolia, Asparagus racemasus, Withania somnifera and Picrorrhiza kurroa markedly inhibited the suppression of chemotactic activity and production of interleukin-1 and tumour necrosis factor induced by the carcinogen ochratoxin in mouse macrophages.

Anti-oxidant activity: [41]

An extract of Tinospora cordifolia reduced the toxicity induced by free radicals and inhibited lipid peroxidation and the generation of superoxide and hydroxyl radicals in vitro. It reduced the toxic side effects of cyclophosphamide in mice as shown by the total white blood cell count, bone marrow cellularity and esterase-positive cells. It also partially reduced elevated lipid peroxides in serum and liver, as well as alkaline phosphatase and glutamine pyruvate transaminase.

Anti-stress activity: [36]

An ethanolic extract of the roots of Tinospora cordifolia normalized stress- induced biochemical changes in nor-epinephrine, dopamine, 5- hydroxytryptamine and S-hydroxyindoleacetic acid levels in experimental rat models.

Anti-ulcer activity: [42]

An ethanolic extract of the roots of Tinospora cordifolia, in combination with Centella asiatica, afforded significant protective action against restraint stress-induced ulcer formation. The activity was comparable to diazepam in rats.

Immunomodulatory activity: [43]

Syringin and cordiol, isolated from Tinospora cordifolia, inhibited the in vitro immuno-haemolysis of antibody-coated sheep erythrocytes by guinea pig serum. This was found to be due to inhibition of C3-convertase in the classic complement pathway. Humoral and cell-mediated immunity were also dose dependently enhanced and an increase in IgG antibody in serum was observed. Cordioside, cordiofolioside and cordiol also activated macrophages significantly.

Hepatoprotective activity: [44]

A study in goats showed an improvement in clinical and haemato-biochemical parameters of the liver, suggesting a protective action for T. cordifolia.

Hypoglycemic activity: [45] [46]

The aqueous, alcoholic and chloroform extracts of the leaves of T. cordifolia exerted a significant hypoglycemic effect in normal and

alloxan- treated rabbits at doses of 50, 100, 150 and 200 mg/kg body weight. Oral administration of an aqueous root extract to alloxan diabetic rats caused a significant reduction in blood glucose and brain lipids, an increase in body weight, hemoglobin and hepatic hexokinase levels and a lowering of hepatic glucose-6-phosphatase, serum acid phosphatase, alkaline phosphatase and lactate dehydrogenase.

3] DRIED EARTH WORM:

BIOLOGICAL NAME : Lampito mauritii.

MEDICINAL USE:

Extracting and using biologically active compounds from earthworms have traditionally been practiced by indigenous people throughout the world, more particularly in Asia, including China, India and Myanmar (Ranganathan 2006). Earthworms have a dense nutritional content because of their soil based origin. The earthworm extract is used in various conditions as anti-inflammatory, anti-oxidant, anti-ulcer and Immunomodulatory drug.

PHARMACOLOGICAL STUDIES:

Various studies indicate that Earthworm extract possesses anti-inflammatory [47], anti-oxidant [48] and anti-ulcer [49] activities.

PHOTOGRAPH OF ECLIPTA PROSTRATA

PHOTOGRAPH OF TINOSPORA CORDIFOLIA

AIM AND OBJECTIVES

AIM AND OBJECTIVES

The present study has been done,

1] To evaluate the analgesic effect of ethanolic extract of Seenthil churanam in mice.

2] To evaluate the anti-inflammatory effect of ethanolic extract of Seenthil churanam in rats.

PLAN OF WORK

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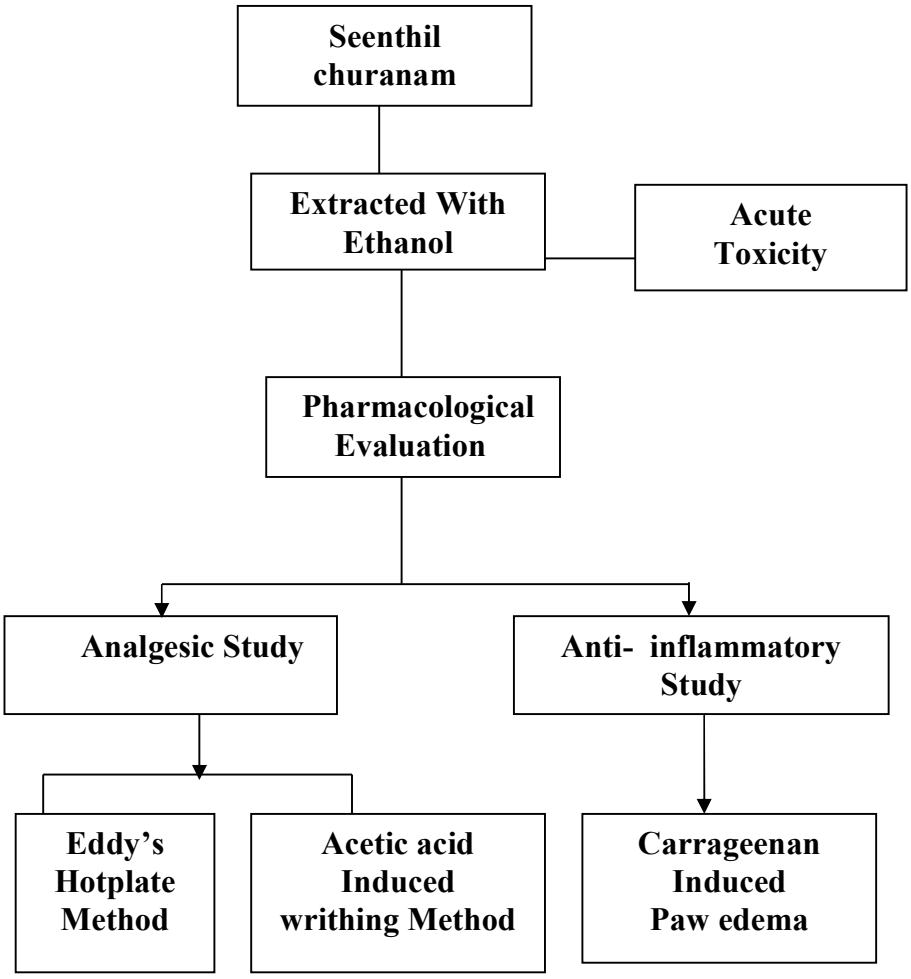
Seenthil churanam
Extracted With Ethanol
Acute Toxicity
Pharmacological Evaluation
Analgesic Study
Anti- inflammatory Study
Eddy's Hotplate Method
Acetic acid Induced writhing Method
Carrageenan Induced Paw edema

MATERIALS AND METHODS

MATERIALS AND METHODS

The present study was carried out in the Department of Pharmacology, Central Research Institute of Siddha, Chennai and in the Institute of Pharmacology, Madras Medical College, Chennai. The study has been designed for the pharmacological evaluation of analgesic and anti-inflammatory activity of Seenthil Churanam.

DRUGS AND CHEMICALS:

Preparation of Ethanolic Extract of Seenthil Churanam :

The polyherbal formulation, Seenthil churanam was purchased from IMPCOPS, Chennai. The extraction process was carried out in Madras Medical College, Chennai. The powder was extracted by cold maceration process using absolute ethanol (99 % v/v). 1000gm of the churanam is macerated in a glass jar with 2000ml of ethanol (99 % v/v) for 7 days with stirring twice a day and change of solvent every 3 days.

The filtrate obtained is concentrated to a semisolid mass. This extract was kept in the dessicator for further solidification. The yield was found to be 10 %. The solidified extract was stored in air tight container in refrigerator. The extract was administered to animals as suspension in Tween 80 (20 % v/v) throughout the experiment.

Tween 80: [50]

Tween 80 is a non-ionic surfactant and emulsifier derived from polyethoxylated sorbitol and oleic acid. It is also called as Polysorbate 80. It is a viscous, water soluble yellow liquid. Tween 80 solution is used in animal experiments as a solvent.

Carrageenan: [51]

Carrageenan is a sulphated polysaccharide obtained from the sea weed [Rhodophyceae] and by causing the release of histamine, serotonin, bradykinin and prostaglandins it produces inflammation and edema.

Analgin:

Used as a Standard drug for evaluating the central analgesic activity of the test drug. Dose of Analgin used was 500mg / kg in mice.

Aspirin:

Used as a Standard drug for evaluating the peripheral analgesic activity of the test drug. Dose of Aspirin used was 100 mg / kg in mice.

Diclofenac:

Used as a Standard drug for evaluating the anti-inflammmatory activity of the test drug. Dose of Diclofenac used was 100 mg / kg in rats.

EXPERIMENTAL ANIMALS:

The present study was conducted after obtaining approval of Institutional Animal Ethical Committee and this protocol met the national guidelines as per the guidelines of CPCSEA [52]. All the animals used in the study were obtained from the animal house of Department of Pharmacology CRIS, Chennai Reg: No: 512/01/a/CPCSEA dated 31/10/2001.

Albino rats:

Young mature adult Albino rats of wistar strain of either sex weighing about 150 – 200 gm were used. The animals were obtained from the inbred colony maintained in CRIS, Chennai.

Swiss albino mice:

Swiss albino mice of both sexes weighing about 25 – 30 gm were used. The animals were obtained from the inbred colony maintained in the CRIS animal house.

APPLIANCES / EQUIPMEMTS:

Oral feeding tube made from 18 gauge bent needle with blunt end. It was fixed to a 1ml glass syringe. The feeding tube was introduced into the mouth of the rat and drugs were administered.

Tuberculin syringe (1ml capacity) used for injecting very small volume of drugs. (for eg.0.1ml of carrageenan into the rat hind paw).

METHODS

The ethanolic extract of Seenthil churanam was initially prepared by Cold Maceration process. Before starting the pharmacological evaluation of ethanolic extract of Seenthil churanam, Toxicity studies were carried out to evaluate the toxicity of Seenthil churanam.

ACUTE TOXICITY STUDIES

Acute Toxicity studies were carried out according to the OECD-423 Guidelines [53]. Three albino rats of either sex weighing between 150 – 200 grams were selected. The animals were subjected to overnight fasting. Only water was allowed during fasting. The extract is suspended in 20 % v/v Tween 80 and administered orally by oral feeding tube. A dose of 2000mg / kg was selected according to the prescribed guidelines and the ethanolic extract of Seenthil churanam was administered as a single dose. Then the animals were deprived of food only for 4 hours after the administration of test drug.

Observation:

The animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily there-after, for a

total of 14 days. The following clinical observations were made and recorded.

1. Toxic signs:

All the rats were observed for any toxic signs.

2. Pre-terminal deaths:

All the rats were observed daily for any pre-terminal deaths.

3. Body weight:

Individual body weight was recorded for all the animals once in a week.

4. Cage side observation:

The home cage activity, faeces amount, faeces colour, faeces consistency and behaviour of the animal were observed once every two days.

5. Physical examination:

In physical examination, the following observations were made, thrice weekly→ Hair coat, respiration rate, respiration character, lacrimation, salivation, eye prominence, eyelid closure, convulsions, biting and tremors were observed.

The locomotor activity, rearing activity, tail elevation, static limb position, abnormal gait, ataxic gait, head position and pinna touch response were monitored twice in a week.

Based on the acute toxicity studies, two doses i.e, 200 mg/kg and 400 mg/kg were selected as doses, according to the guidelines for further studies.

EVALUATION OF ANALGESIC ACTIVITY OF ETHANOLIC EXTRACT OF SEENTHIL CHURANAM:

Two methods were used for the evaluation of analgesic activity of ethanolic extract of Seenthil churanam. They are,

- ACETIC ACID INDUCED WRITHING TEST
- EDDY'S HOT PLATE METHOD

1) **ACETIC ACID INDUCED WRITHING TEST**: [54] [56]

It is a sensitive test used for evaluating the peripheral analgesic activity of a drug. Pain is induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called writhing. The test is suitable to detect analgesic activity although some psychoactive agents also show activity. An irritant such as phenylquinone or acetic acid is injected intraperitoneally to mice and the stretching reaction is evaluated. The

reaction is not specific for the irritant. Writhings were induced by injecting acetic acid into the peritoneal cavity of mice.

Experimental animals and dosage:

24 swiss albino mice of either sex with a weight between 20-30g were selected. The animals were divided into four groups, six mice per group.

Group 1: Control group.

Animals received 0.2ml of 20% Tween 80 which was the vehicle.

Group 2: Standard drug group.

Animals received 100mg / kg of Aspirin.

Group 3: Test drug group.

Animals received 200mg / kg of Ethanolic extract of Seenthil churanam.

Group 4: Test drug group.

Animals received 400mg / kg of Ethanolic extract of Seenthil churanam.

Experimental procedure:

Both the standard and the ethanolic extract of Seenthil churanam was suspended in 20 % v/v Tween 80 and administered orally, by gavage. All the animals were deprived of food but allowed free access to water for 4 hours before the procedure.

The vehicle, standard drug Aspirin (100mg / kg) and the ethanolic extract of Seenthil churanam (200mg / kg and 400mg / kg)were administered orally to the animals of the respective groups after the fasting period of 4 hours.

Half an hour after the treatment, the mice were injected intraperitoneally with 0.2ml of 0.6 % acetic acid solution to induce the writhing. The mice were then observed for writhing between 5 and 15 minutes after acetic acid injection and the number of writhing movements were recorded for each animal. A writhe is defined as a stretch torsion to one side, drawing up of the hind legs, retraction of the abdomen, so that the belly of the mouse touches the floor. The response of the extract and Aspirin treated groups were compared with those of the animals in the control groups (0.2 ml of 20 % Tween 80).

The Percentage Inhibition of writhings was calculated by the formula,

$$\frac{Wc - Wt}{Wc} \times 100$$

where, Wc - writhes in Control

Wt - writhes in Test.

Statistical Analysis:

Data were expressed as mean ± SE. The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The values were expressed as mean ± SEM and $p < 0.05$ was considered significant.

2) **EDDY'S HOT PLATE METHOD**: [55] [56]

It is a commonly used method for testing the central analgesic activity of a drug. The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics, whereas peripheral analgesics of the acetylsalicylic acid or phenyl-acetic acid type do not generally affect these responses. The Hotplate which is commercially available consists of an electrically heated surface. The temperature is controlled for 55° to 56 °C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch.

Experimental animals and dosage:

Twenty four Swiss mice of either sex weighing between 20 – 30g were taken and the animals with the cut off time of below 10 sec were

selected. The animals were divided into four groups of six mice per group.

Group 1: Control group:

Animals received 0.2ml of 20% Tween 80 which was the vehicle.

Group 2: Standard drug group:

Animals received 500 mg / kg of Analgin.

Group 3: Test drug group:

Animals received 200 mg / kg of Ethanolic extract of Seenthil churanam.

Group 4: Test drug group:

Animals received 400 mg / kg of Ethanolic extract of Seenthil churanam.

Experimental procedure:

Both the standard and the ethanolic extract of seenthil churanam is suspended in 20 % v/v Tween 80 and administered orally, by gavage. All the animals were deprived of food but allowed free access to water for 4 hr before the procedure.

The reaction time i.e. the time between placing the animal on the hot plate and the jumping of animals or the beginning of licking of fore paw or hind paw by the animals was recorded for all the mice. Not more than 30 seconds was allowed on the hot plate to avoid any thermal injury to the animal. Then the animals were divided into four groups. The vehicle, standard drug Analgin 500mg/ kg and the ethanolic extract of Seenthil churanam 200mg/ kg and 400 mg/ kg were administered orally to the animals of the respective groups after the fasting period of 4 hours. The reaction time was noted 30 minutes after drug administration and the results were tabulated.

Percentage Increase of reaction time was calculated by the formula,

$$\frac{Rt - Rc}{Rc} \times 100$$

where, Rt - Reaction time in test group

Rc - Reaction time in control group.

Statistical analysis:

Datas were expressed as mean ±SE.

The results were statistically analyzed by the One-way repeated measures ANOVA followed by TUKEY multiple comparison test. $P < 0.05$ versus respective control was taken as significant.

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF SEENTHIL CHURANAM:

Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent. Many phlogistic agents (irritants) have been used, such as brewer's yeast, formaldehyde, dextran, egg albumin, kaolin, Aerosil, sulfated polysaccharides like carrageenin or naphthoylheparamine. Usually, the volume of the injected paw is measured before and after application of the irritant and the paw volume of the treated animals is compared with the controls.

Many methods have been described how to measure the paw volume by simple and less accurate and by more sophisticated electronically devised methods. The value of the assessment is less dependent on the apparatus but much more on the irritant being chosen. Some irritants induce only a short lasting inflammation whereas other irritants cause the paw edema to continue over more than 24 h. In this study, the method employed for the study of anti-inflammatory activity was, Carrageenan induced paw edema method.

CARRAGEENAN INDUCED RAT-PAW EDEMA METHOD: [57] [58]

The anti-inflammatory activity was assessed by carrageenan Induced paw edema method. The acute inflammatory edema was induced by injecting 1% carrageenan into the sub plantar surface of hind paw of the rat. The increase of paw volume is calculated as percentage compared with the volume measured immediately after injection of the irritant for each animal. Effectively treated animals show much less edema. The difference of average values between treated animals and control groups is calculated for each time interval and statistically evaluated. The differences at the various time intervals give some hints for the duration of the anti-inflammatory effect. The Apparatus used in this study for recording the rat paw edema is the Digital Plethysmograph.

Percetage Inhibition of Inflammation was calculated by the formula,

$$\frac{Vc - Vt}{Vc} \times 100$$

where Vc – Volume of paw edema in Control group

Vt - Volume of paw edema in Test group.

Experimental animals and dosage:

Twenty four Albino rats of either sex weighing between 150-200 grams were divided into 4 groups of 6 animals each.

Group 1: control group:

Animals received 0.2 ml of 20% Tween 80.

Group 2: standard group:

Animals received Diclofenac in a dose of 100 mg / kg.

Group 3: Test drug group:

Animals received 200 mg/ kg of ethanolic extract of Seenthil churanam.

Group 4: Test drug group:

Animals received 400 mg/ kg of ethanolic extract of Seenthil churanam.

Experimental procedure:

One hour after the oral administration of control drug, standard drug and the test drug to respective groups, a sub-plantar injection of 0.1ml of 1% carrageenan was administered into the right hind paw of each rat in the respective group. A mark was made on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The right hind paw volume was measured by using the Digital plethysmograph immediately (zero hour volume) and after each hour for four hours. The difference between the paw volumes at zero hour and at each hour

indicated the actual edema. Thus the paw edema volume in animals treated with drugs in group wise was compared with that in the untreated control group.

Statistical analysis:

Datas were expressed as mean ± SE. The results were statistically analyzed by One-way repeated measures ANOVA followed by TUKEY multiple comparison tests. $P < 0.05$ was considered as significant.

RESULTS

RESULTS

The ethanolic extract of Seenthil churanam was studied for toxicity and pharmacological evaluation of analgesic and anti-inflammatory activity. The results of the study on the 4 groups of animals were observed and analyzed by suitable statistical methods.

ACUTE TOXICITY STUDY:

The ethanolic extract of Seenthil churanam did not show any toxic effects in the study. All the animals very well tolerated the churanam and there was no mortality.

ANALGESIC EFFECT:

Pain induced by thermal stimulus was assessed by Hot plate method and that induced by chemical stimulus was assessed by Acetic acid induced writhing method. The effects of Tween 80 used as Vehicle was studied (control) and compared with the effects produced by Aspirin, Analgin and 2 doses of Seenthil churanam (200mg/kg and 400mg/kg).

THERMAL STIMULUS - HOT PLATE METHOD:

Group 1 - The observations of the reaction time in this group was not altered proving that Tween 80 had no analgesic effect.

Group 2 - The observations of the reaction time in this group which received Analgin (standard) was increased. The P value of 0.001 was obtained which is statistically significant.

Group 3 - The observations of the reaction time in this group which received ethanolic extract of Seenthil churanam (200mg/kg) was increased. The onset of analgesic effect was seen at 60 minutes and the peak effect was seen at 180 minutes after the drug administration. The P value of 0.001 was obtained which is statistically significant.

Group 4 - The observations of the reaction time in this group which received ethanolic extract of Seenthil churanam (400mg/kg) was increased. The onset of analgesic effect was seen at 90 minutes and the peak effect was seen at 180 minutes after the drug administration. The P value of 0.001 was obtained which is statistically significant.

Table 1:

Effect of Seenthil churanam on Hot plate method

Treatment	Initial response Time (Sec)	Mean reaction time in seconds (mean ± SEM)					
		30 min	60 min	90 min	120 min	150 min	180 min
vehicle control	5.58 ± 0.75	5.07 ± 0.72	4.9 ± 0.69	5.27 ± 0.84	5.52 ± 0.71	5.9 ± 0.74	5.98 ± 0.72
Analgin (500 mg/kg)	4.6 ± 0.16	11.82 ± 1.41*	13.97 ± 1.11*	10.52 ± 0.88*	12.57 ± 0.78*	8.5 ± 0.67*	7.8 ± 0.50*
Seenthil churanam (200mg/kg)	4.46 ± 0.73	12.57 ± 0.56*	10.83 ± 0.62* #	13.73 ± 0.88* #	11.58 ± 0..43*	10.7 ± 0.3* #	8.7 ± 1.47* #
Seenthil churanam (400mg/kg)	6.13 ± 0.65	13.5 ± 1.12*	12.41 ± 0.77*	14.43 ± 0.48* #	12.66 ± 0.64*	11.65 ± 0.42* #	9.93 ± 0.21* #

* $p<0.001$, (significant difference) as compared with vehicle control.

# $p<0.05$, (significant difference) as compared with Standard (Analgin).

Table 1 shows

Effect of Seenthil churanam by Hot plate method in mice.

Analgin (500mg/kg) showed a significant increase in the mean reaction time when compared to control.

Seenthil churanam at both doses significantly increased the mean reaction time of mice when compared to control and standard group.

Figure 1:

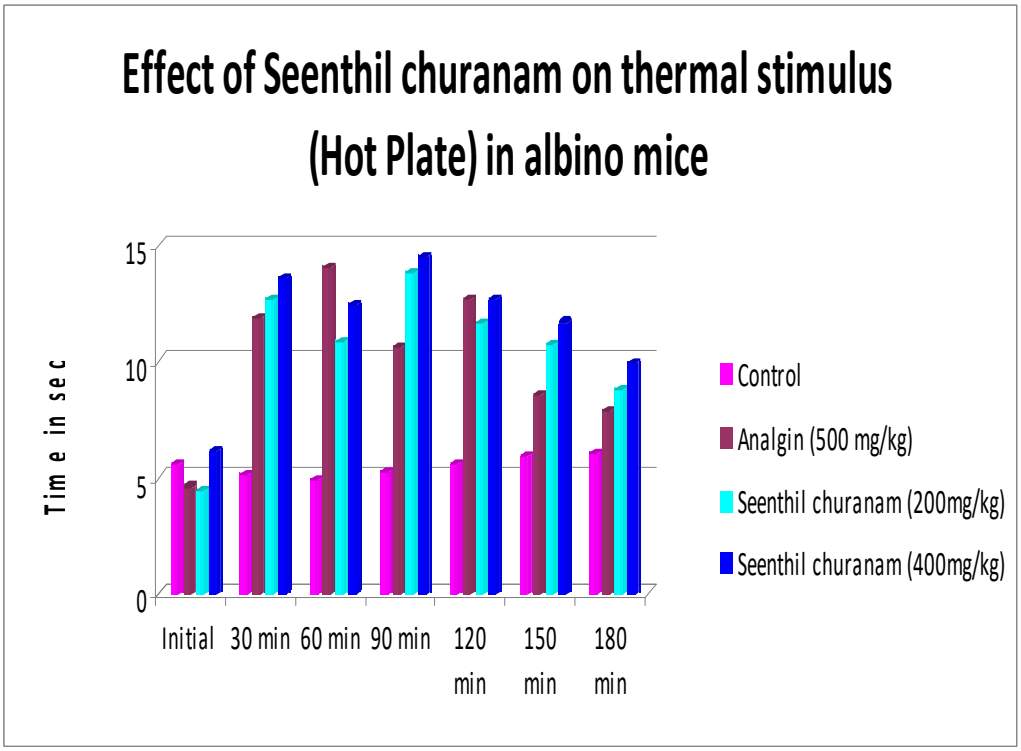

Figure 1 is the graphical representation of Table 1.

Figure 2:

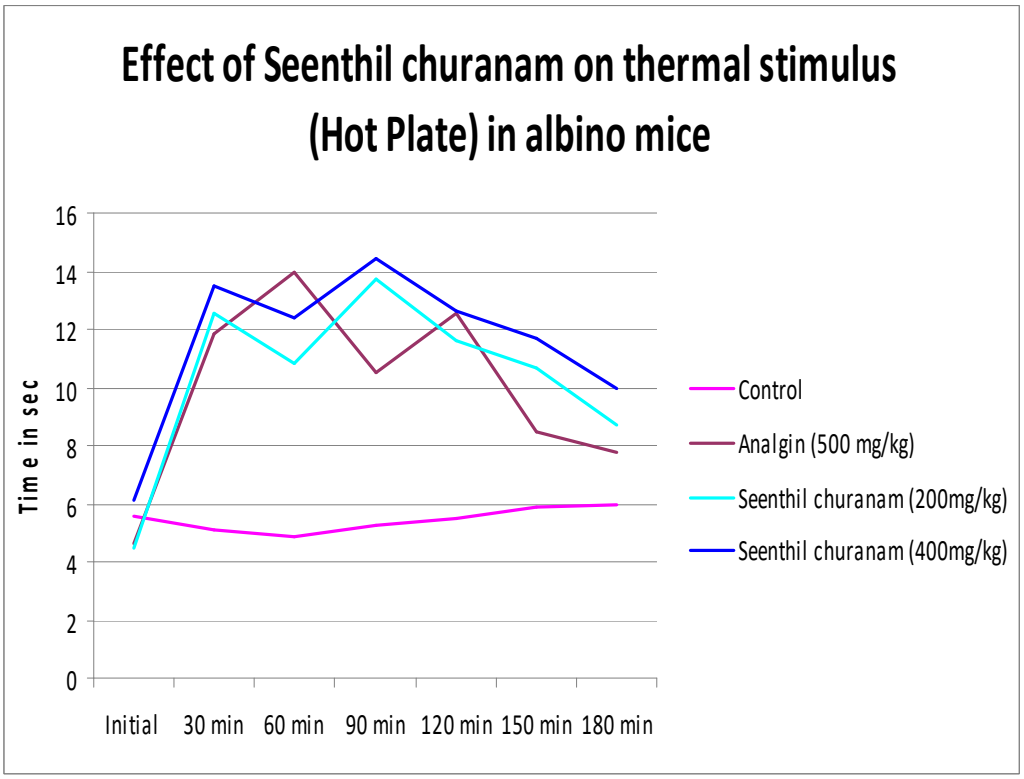

Figure 2 is the graphical representation of Table 1.

Table 2:

Percentage increase of reaction time in albino mice (Hot plate method)

Group		% Increase of reaction time
Group 2	Analgin (500mg/kg)	124
Group 3	Seenthil churanam (200mg/kg)	110
Group 4	Seenthil churanam (400mg/kg)	130

Table 2 shows

Percentage increase of reaction time in albino mice.

Seenthil churanam showed a significant increase in reaction time when compared to both control and standard.

The analgesic effect of Seenthil churanam (400mg/kg) was better than Seenthil churanam (200mg/kg) and standard.

Figure 3:

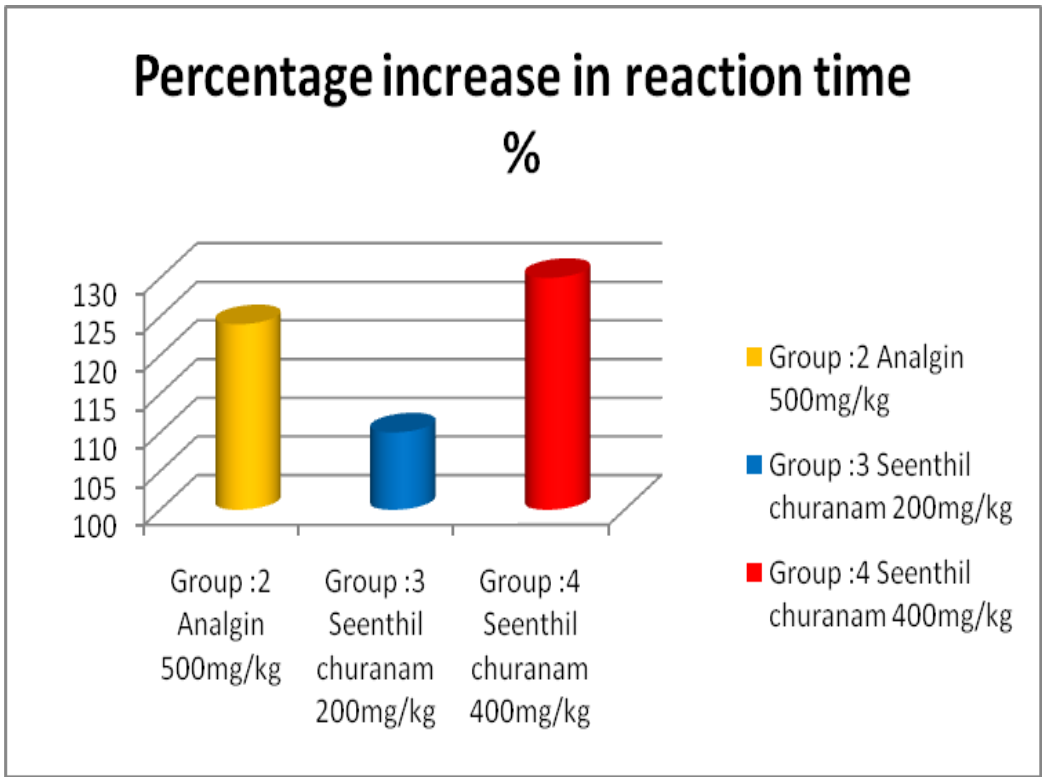

Figure 3 is the graphical representation of Table 2.

CHEMICAL STIMULUS – ACETIC ACID INDUCED WRITHING

METHOD:

Group 1 - The observation of the mean number of writhings in this group was not altered proving that Tween 80 had no analgesic effect.

Group 2 - The observation of the mean number of writhings in this group which received Aspirin (standard) was decreased. The P value of 0.001 was obtained which is statistically significant.

Group 3 - The observation of the mean number of writhings in this group which received Seenthil churanam (200mg/kg) was decreased. The P value of 0.001 was obtained which is statistically significant.

Group 4 - The observation of the mean number of writhings in this group which received Seenthil churanam (400mg/kg) was decreased. The P value of 0.001 was obtained which is statistically significant

Table 3:

Effect of Seenthil churanam on acetic acid induced writhing in albino mice

Group	No.of writhings (Mean±SE)
Group :1 Control	43.83±1.30
Group :2 Aspirin 100mg/kg	8.33±2.51 *
Group :3 Seenthil churanam 200mg/kg	31 ± 3.61 *
Group :4 Seenthil churanam 400mg/kg	21 ± 1.41 *

* $p < 0.001$, (significant difference) when compared to control.

Table 3 shows

Effect of Seenthil churanam on acetic acid induced writhing in mice.

Seenthil churanam at both doses significantly decreased the number of writhings when compared to control.

Seenthil churanam at both doses showed a p value of <0.001 which is statistically significant.

The analgesic effect of Seenthil churanam (400mg/kg) was better when compared to Seenthil churanam (200mg/kg).

Figure 4:

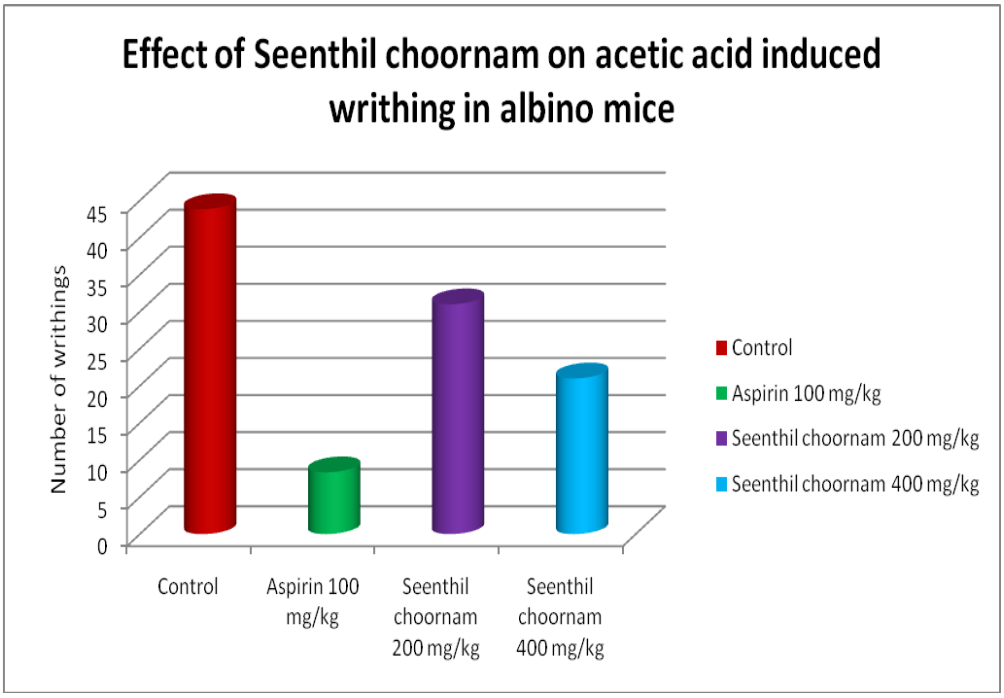

Figure 4 is the graphical representation of Table 3.

Table 4:

Percentage inhibition of writhing in albino mice

Group	%inhibition
Group :2 Aspirin 100mg/kg	80.99
Group :3 Seenthil churanam 200mg/kg	29.28
Group :4 Seenthil churanam 400mg/kg	52.09

Table 4 shows

Percentage inhibition of writhing in albino mice.

Seenthil churanam at both doses showed a significant percentage inhibition of writhings when compared to both control and standard drug.

The percentage inhibition of writhings by Seenthil churanam (400mg/kg) was better when compared to Seenthil churanam (200mg/kg).

Figure 5:

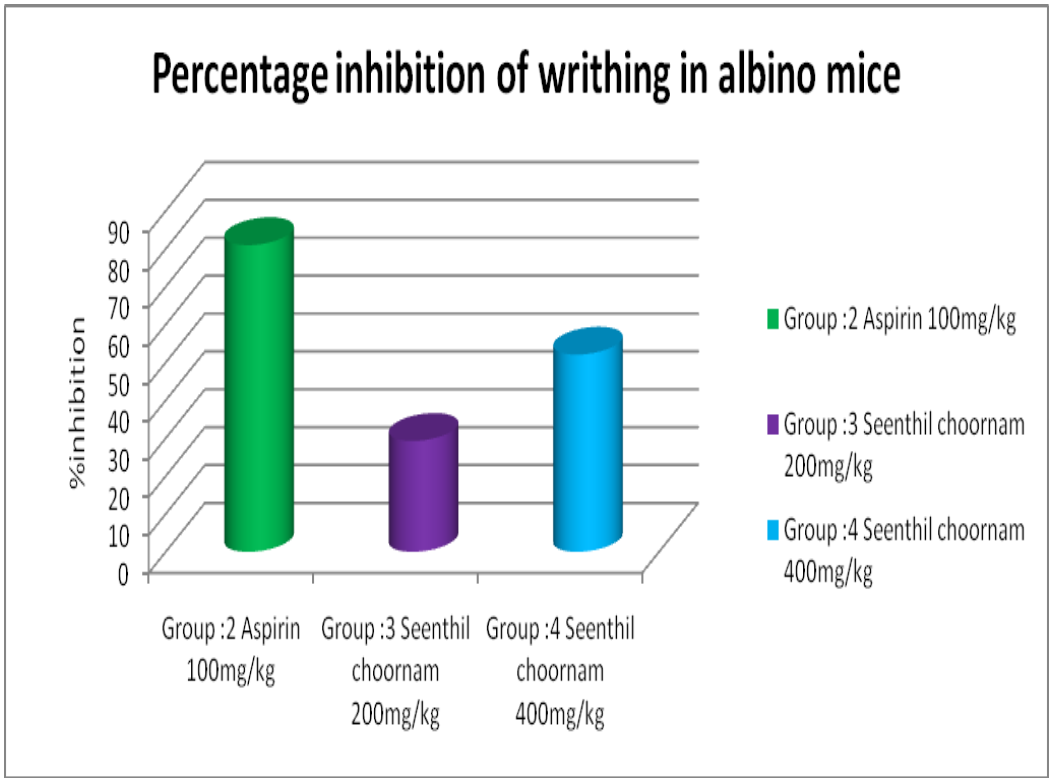

Figure 5 is the graphical representation of Table 4.

ANTI-INFLAMMATORY EFFECT:

The anti-inflammatory effect was studied by Carrageenan induced paw edema method. The effects of Tween 80 used as Vehicle was studied (control) and compared with the effects produced by Diclofenac and 2 doses of Seenthil churanam (200mg/kg and 400mg/kg).

CARRAGEENAN INDUCED RAT PAW EDEMA METHOD:

Group 1 - The observation of the mean paw edema volume in this group was increased proving that Tween 80 had no significant anti- inflammatory effect.

Group 2 - The observation of the mean paw edema volume in this group which received Diclofenac (standard) was decreased. The P value of <0.05 was obtained which is statistically significant.

Group 3 - The observation of the mean paw edema volume in this group which received Seenthil churanam (200mg/kg) was decreased. The P value of <0.05 was obtained which is statistically significant.

Group 4 - The observation of the mean paw edema volume in this group which received Seenthil churanam (400mg/kg) was decreased. The P value of <0.05 was obtained which is statistically significant.

Table 5:

Effect Of Seenthil Churanam On Carrageenan Induced Paw Edema In Rats					
Groups	**Initial paw volume (ml)**	**Increase in paw volume (ml) Mean ± S.E**			
		1st hr	2nd hr	3rd hr	4th hr
Control	1.21 ± 0.013	0.465 ± 0.03	1.00 ± 0.07	0.952 ± 0.03	0.94 ± 0.05
Diclofenac 100mg/kg	1.207± 0.016	0.33 ± 0.03*	0.103 ± 0.013*	0.063 ± 0.011*	0.05 ± 0.02*
Seenthil churanam 200mg/kg	1.198 ± 0.009	0.425 ± 0.04*	0.33 ± 0.04*	0.26 ± 0.04*	0.25 ± 0.05*
Seenthil churanam 400mg/kg	1.19 ± 0.01	0.45 ± 0.01*	0.26 ± 0.02*	0.17 ± 0.02*	0.11 ± 0.03*

* $p < 0.05$ significant when compared to control.

Table 5 shows

Effect of Seenthil churanam on carrageenan induced paw edema in rats.

Diclofenac showed a significant decrease in the paw edema volume when compared to control and Seenthil churanam.

Seenthil churanam at both doses also significantly decreased the paw volume when compared to control and standard.

Seenthil churanam at both doses showed a p value of <0.05 which was considered statistically significant.

Figure 6:

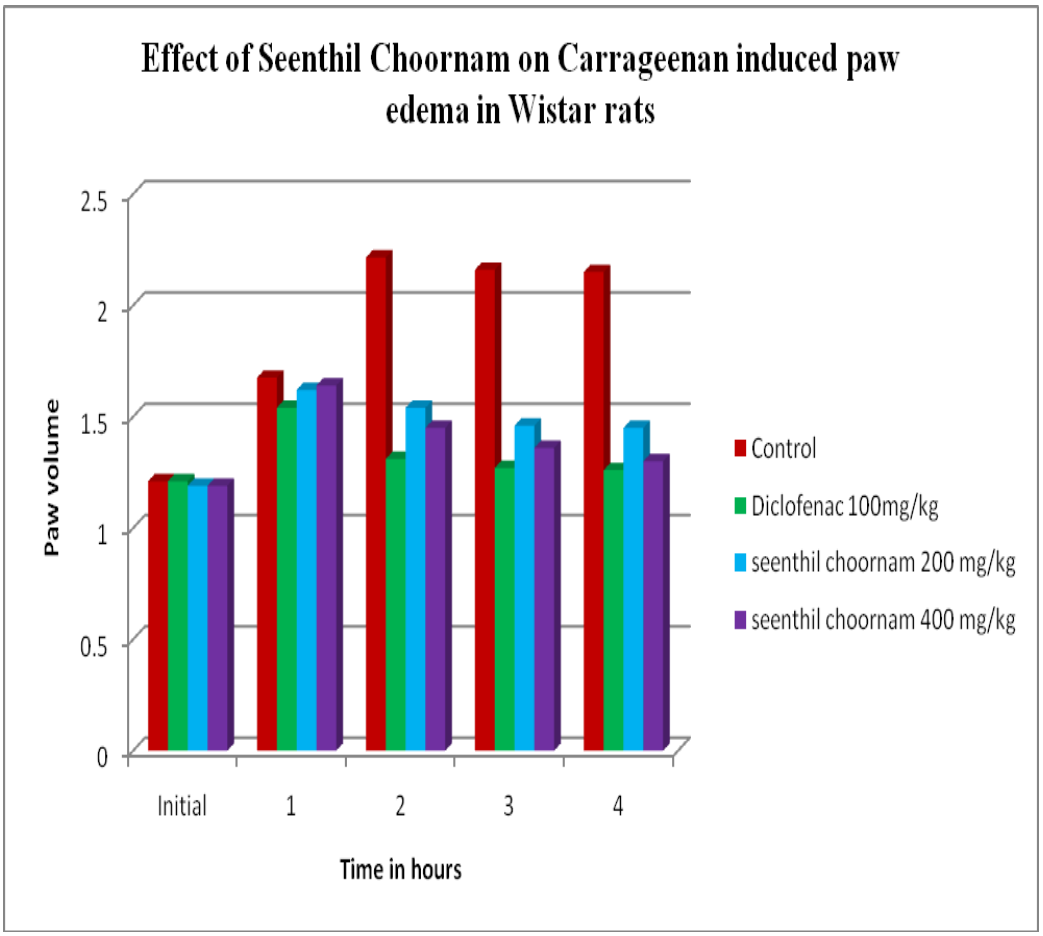

Figure 6 is the graphical representation of Table 5.

Figure 7:

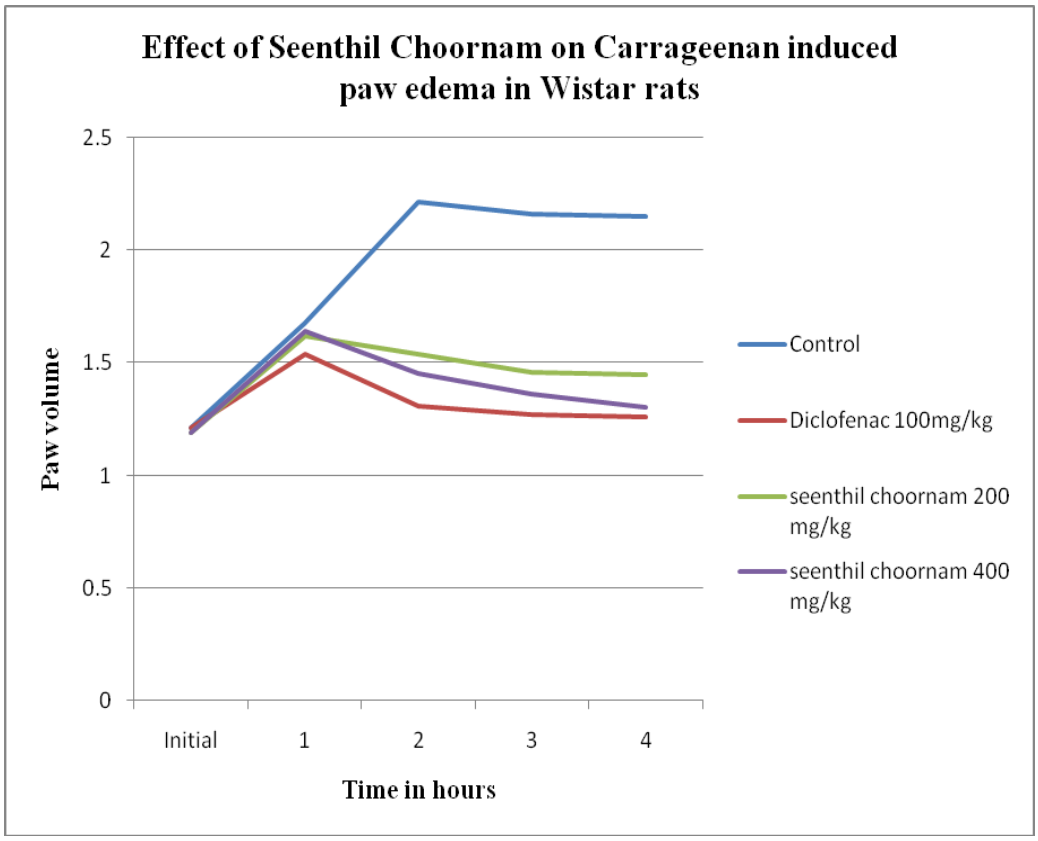

Figure 7 is the graphical representation of Table 5.

Table 6:

Percentage inhibition of inflammation	
TREATMENT	**% INHIBITION**
Diclofenac 100mg/kg	94.68
Seenthil churanam 200 mg/kg	73.4
Seenthil churanam 400 mg/kg	88.29

Table 6 shows

Percentage inhibition of inflammation by Seenthil churanam.

Diclofenac showed a significant percentage of inhibition of inflammation when compared to control and Seenthil churanam.

Seenthil churanam at both doses also showed a significant percentage of inhibition of inflammation when compared to control.

The anti-inflammatory effect of Seenthil churanam (400mg/kg) was better than when compared to Seenthil churanam (200mg/kg).

Figure 8:

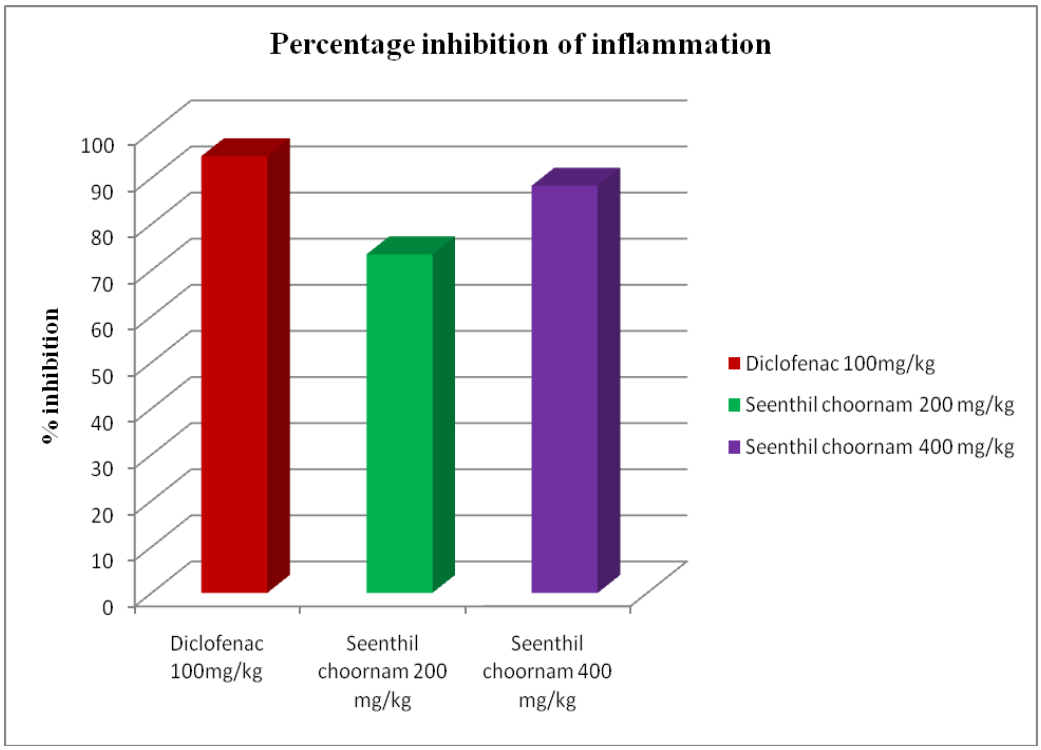

Figure 8 is the graphical representation of Table 6.

DISCUSSION

DISCUSSION

Seenthil churanam is a polyherbal formulation used in Siddha medicine. The ingredients of Seenthil churanam are whole plant extracts of Eclipta prostata, Tinospora cordifolia and the dried powder form of Earthworm.

Eclipta prostata [29] is a well known medicinal plant widely distributed in several countries. It has been claimed to possess analgesic [32], anti-inflammatory [33] and Immunomodulatory [31], hypolipedemic [35], properties. It is also used as antisnake-venom [34] for the treatment of snakebites in various parts of the world.

Tinospora cordifolia [29] is also used as an analgesic [36], anti-inflammatory [37] and anti-allergic [38] in various conditions. It has been claimed to possess anti-cancer [40], anti-ulcer [42], immunomodulatory [43], hepatoprotective [44] and hypoglycemic [45] activities. Dried powder extract of Earthworm is used for its anti-inflammatory [47] and anti-oxidant [48] properties.

Though the individual components of Seenthil churanam had been already studied for various medicinal properties, there is no established documentation of the beneficial effects of Seenthil churanam as a whole polyherbal formulation. Hence this study was undertaken to evaluate the

analgesic and anti-inflammatory properties of Seenthil churanam by suitable methods in animals.

In the present study, ethanolic extract of Seenthil churanam was evaluated for analgesic and anti-inflammatory activity. Initially ethanolic extract was prepared by Cold Maceration Process. Then Toxicity studies were carried out to evaluate the toxicity of ethanolic extract of Seenthil churanam. A single dose of 2000mg/kg was administered orally to the animals and the animals were observed for a period of 24 hours for any mortality or any toxic adverse effects. Then the animals were followed up for a period of 14 days. The following parameters were observed - Toxic signs, Preterminal deaths, Body weight, Cage side observation and Physical examination.

The observation from the toxicity studies showed that ethanolic extract of Seenthil churanam was well tolerated by the animals. There was no major adverse effect and there was no mortality. This was similar to the study conducted with Eclipta prostata, Tinospora cordifolia and Earthworm where, no toxic effects were reported. Based on the acute toxicity studies, 2 doses of ethanolic extract of Seenthil churanam (200mg/kg and 400mg/kg) were seleced and then the pharmacological evaluation of ethanolic extract of Seenthil churanam for analgesic and anti-inflammaory activities was carried out.

The analgesic activity of ethanolic extract of Seenthil churanam was studied by Hotplate method [55] and Acetic acid induced writhing method [54]. Hotplate method determines the central analgesic activity, whereas the Acetic acid induced writhing method determines the peripheral analgesic activity of the test drug. In the Hotplate method, both the standard (Analgin) and the 2 doses of ethanolic extract of Seenthil churanam showed significant analgesic activity when compared to control. The ethanolic extract of Seenthil churanam (400mg/kg) showed better analgesic activity than ethanolic extract of Seenthil churanam (200mg/kg) and Analgin.

In the Acetic acid induced writhing method, both the standard (Aspirin) and the 2 doses of ethanolic extract of Seenthil churanam showed significant analgesic activity when compared to control. The analgesic activity of ethanolic extract of Seenthil churanam (400mg/kg) was better than that of Seenthil churanam (200mg/kg). However, the analgesic activity of ethanolic extract of Seenthil churanam was not equal to that of Aspirin in this method.

The anti-inflammatory activity of ethanolic extract of Seenthil churanam was studied by Carrageenan induced rat paw edema method [57]. This experimental model has been given greater emphasis over the years, because edema induced by Carrageenan is reported to have been inhibited by majority of the established anti-inflammatory agents. Moreover the

lesions induced by Carrageenan were said to resemble histologically those of Rheumatoid arthritis in humans to a certain extent.

In the Carrageenan induced paw edema method, both standard (Diclofenac) and the 2 doses of ethanolic extract of Seenthil churanam showed significant anti-inflammatory activity. The ethanolic extract of Seenthil churanam (400mg/kg) showed better anti-inflammatory activity than that of Seenthil churanam (200mg/kg). However, the anti-inflammatory activity of ethanolic extract of Seenthil churanam was not equal to that of Diclofenac in this method.

CONCLUSION

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From our study, we can conclude that,

1) The ethanolic extract of Seenthil churanam has significant analgesic activity.

2) The ethanolic extract of Seenthil churanam also has significant anti- inflammatory activity.

3) The ethanolic extract of Seenthil churanam did not show any toxic effects in the study.

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